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The characterization of murine BCMA gene defines it as a new member of the tumor necrosis factor receptor superfamily

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Abstract

The BCMA gene is a new gene discovered by the molecular analysis of a t(4;16) translocation, characteristic of a human T cell lymphoma. It has no significant similarity with any known protein or motif, so that its function was unknown. This report describes the cloning of murine BCMA cDNA and its genomic counterpart. The mouse gene is organized into three exons, like the human gene, and lies in murine chromosome 16, in the 16B3 band, the counterpart of the human chromosome 16p13 band, where the human gene lies. Murine BCMA cDNA encodes a 185 amino acids protein (184 residues for the human), has a potential central transmembrane segment like the human protein and is 62% identical to it. The murine BCMA mRNA is found mainly in lymphoid tissues, as is human BCMA mRNA. Alignment of the murine and human BCMA protein sequences revealed a conserved motif of six cysteines in the N-terminal part, which strongly suggests that the BCMA protein belongs to the tumor necrosis factor receptor (TNFR) superfamily. Human BCMA is the first member of the TNFR family to be implicated in a chromosomal translocation.

Introduction

The tumor necrosis factor (TNF)-related cytokines form a large family of pleiotropic mediators of host defense and immune system regulators. They act locally through cell-to-cell contact, when they are integral membrane proteins, or on distant target cells as secreted proteins. There are TNF receptors (TNFR) that mediate the action of TNF-related cytokines leading to cell death or cell proliferation and differentiation (1,2). Most of the genes for TNFR encode type I transmembrane proteins with an extracellular ligand-binding domain, a single membrane-spanning region and a cytoplasmic region that activates cell functions (3). Analysis of the crystal structure of the first three domains of TNFRI, complexed to its ligand, showed that the canonical motif of the TNFR lies in the N-terminal extracellular part of the molecule and contains six cysteine residues (4,5) which form a double loop of ~40 amino acids. The six-cysteine motif forms a twisted ladder consolidated by three intradomain disulfide bridges. However, the C-terminal half of the cysteine motif can form different disulfide connections and topology, as shown by the way the fourth motif of TNFRI (Cys3–Cys6 and Cys4–Cys5) differs from the first three (Cys3–Cys5 and Cys4–Cys6) (6). It has been suggested that the cysteine motif is in fact constructed from two small modules, each having distinctive structural roles (7). The classical first three motifs of TNFRI are A1–B2 pairs whereas the fourth motif is a A1–C2 pair, B2 and C2 being different, both with two disulfide bonds, but with different connections.

The cysteine motif is repeated in all TNFR except one (8),

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which has only one motif. In contrast to the extracellular part of the receptors, the sequences of the cytoplasmic tails are generally dissimilar and none possesses sequences implying catalytic activity. However, several motifs in the C-terminal part of TNFR have been shown to bind, upon ligand binding and receptor trimerization, protein factors transducing the signal. One of these motifs, the 'death domain', is present in TNFRI, Fas, DR3, DR4 and DR5. It is responsible for the capacity of these receptors to induce apoptosis (9,10). A second group of motifs binds signal transducers, TNFR-associated factors (TRAF), that have anti-apoptotic effects; these transducers interact directly with several TNFR, like TNFRII, CD40, CD30 and LtβR (11–14). TRAF2, 5 and 6 mediate the activation of the transcriptional factor NF-κB (15–17), while TRAF2 also activates the c-Jun N-terminal protein kinase (18). The distinct intracellular domains of the TNFR explain why there are so many TNFR, each of which can initiate a distinct signal pathway, depending on the type of cytoplasmic signal transducers recruited by the cytoplasmic domain. Several TNFR have been described recently, some of them are soluble proteins of viral origin, that are believed to block the action of TNF (19–22).

We have previously reported the molecular analysis of a t(4;16)(q26;p13) chromosomal translocation, that occurred in a human malignant T cell lymphoma. The breakpoints of both chromosome partners involved the IL-2 gene on chromosome 4 and a new gene called BCMA, for B cell maturation, on chromosome 16, resulting in the transcription of hybrid IL-2–BCMA mRNA. The human BCMA gene is expressed preferentially in mature B lymphocytes as a 1.2 kb mRNA, which encodes a 184 amino acid peptide (BCMAp) (23). RNase protection analysis using human malignant B cell lines characteristic of different stages of B lymphocyte differentiation demonstrated that the BCMA gene was poorly expressed at the pre-B cell stage and that expression gradually increases as the B lymphocyte matures (24). Polyclonal antibodies were used to show that BCMAp is a non-glycosylated integral membrane protein, which becomes inserted into canine microsomes, as a type I integral membrane protein *in vitro*. BCMAp is not primarily a cell surface protein in human malignant myeloma cell lines, but lies in a perinuclear structure which partially overlaps the Golgi apparatus (25). We have looked for the function of BCMAp by trying to identify similarities with known proteins using the FASTA (26) and BLAST (27,28) algorithms. We found no significant similarities or known protein motifs in the PROSITE data base (29). We therefore cloned and characterized the murine homolog of the BCMA gene, so that the information provided by the conserved parts of the protein could provide clues to the function of BCMA.

This report describes the isolation and sequencing of a murine BCMA cDNA and shows that the murine BCMA gene encodes a 185 amino acid protein containing an internal transmembrane segment. Sequence comparison revealed that six cysteine residues are conserved in the N-terminal part of both human and murine proteins. A sensitive method of sequence analysis, hydrophobic cluster analysis (HCA) (30), indicates that these residues form a cysteine repeat motif found in the extracellular part of TNFR. We therefore suggest that human and murine BCMAp are new TNFR, having a single cysteine-rich motif. The human BCMA gene is the first TNFR gene that is implicated in chromosome translocation.

Methods

Nucleic acid analysis, cloning and sequencing

Genomic DNA extraction, agarose gel electrophoresis, Southern blot transfer and hybridization were performed as previously described (31). Total RNA was extracted from mouse organs or cultured cells using the guanidinium isothiocyanate method (32). Probes were labeled with ^{32}P-labeled nucleotide (Amersham, Paris, France) by the random prime method (33). Poly(A)$^+$ mRNA was prepared using oligo(dT) columns. cDNA libraries were constructed with poly(A)$^+$ mRNAs, using a cDNA synthesis kit (Pharmacia, Orsay, France). The resulting cDNAs were ligated to *Eco*RI-digested λZAPII phage arms (34) and packaged using an *in vitro* packaging kit (Amersham). A 0.7 kb *Sphl–Eco*RI fragment of the human BCMA cDNA was used as probe for blots or to screen the mouse cDNA library under low stringency conditions (washings in 2×SSC, 0.1% SDS solution at room temperature for 30 min). Recombinant phages were cloned as previously described (31). Genomic fragments were subcloned in pUC18 (35) and pBluescript (34,36). cDNA and genomic plasmid inserts were sequenced on both strands by the dideoxy chain termination procedure (37). Oligonucleotides were purchased from Genset (Paris, France). Autoradiographies were made using Kodak XAR5 films at –80°C.

RNase protection assay

Relevant cDNA restriction fragments were subcloned into pGEM-4 plasmid vector. [α-^{32}P]UTP-labeled RNA probes were synthesized from linearized DNA templates by T7 or SP6 RNA polymerase (38) using the Riboprobe II core system kit (Promega Biotech, Madison, WI). Test RNAs (10–30 μg) were hybridized overnight at 56°C with the radiolabeled antisense RNA (3×10^5 c.p.m.) and denatured for 5 min at 90°C (39). The samples were assayed for RNase protection using the RPAII kit under the conditions recommended by the manufacturer (Ambion, Austin, TX). The samples were then precipitated with ethanol and analyzed by electrophoresis through a 5% 'Hydrolink long ranger' (AT Biochemicals, FMC, Rockland, ME) polyacrylamide–7 M urea denaturing gel and autoradiography.

Isolation of cDNA clones by anchor PCR

A modification of the SLIC (single-strand ligation to single-stranded cDNA) method (39,40) was used to obtain the 5′ and 3′ ends of BCMA mRNA. The Marathon cDNA amplification kit (Clontech) was used to obtain BCMA cDNA from 1 μg of poly(A)$^+$ mRNA of J558 plasmacytoma cell line. Fragments were amplified using the primers furnished by the manufacturer and the BCMA-specific primers: 5′BCMA1 (5′-CAGTAAGGCTGACAGGTTGCAG-3′ and 3′BCMA1 (5′-GC-AAGTCAAGTGTGCCAACTGCT-3′). A secondary 'nested' PCR was done using the furnished primers and the specific BCMA ones, 5′BCMA2: 5′-GGAATTCACACGGTTTGCA-

AGCATGCAGC-3′ and 3′BCMA2: 5′-GGAATTCGATGGA-GAAGCCAACTCACACTAG-3′. The amplified products were digested by *Sma*I and *Eco*RI restriction enzymes, subcloned in a pUC 18 vector, and further characterized by sequencing at least 10 different clones for each 5′ and 3′ end.

In vitro transcription and translation

The entire coding sequence of murine and human BCMA cDNAs were cloned under the control of the SP6 polymerase promoter in the *Eco*RI site of the pGEM-4 plasmid. The resulting plasmids were transcribed and translated *in vitro* in the presence of [^{35}S]methionine using the TnT simultaneous transcription and translation kit (Promega Biotech). Translation products were denatured by incubation for 4 min at 95°C in sample buffer (25 mM Tris, pH 6.8, 1 mM EDTA, 5% glycerol, 1% β-mercaptoethanol, 4% SDS and 0.005% bromophenol blue) and analyzed by SDS–PAGE on 12.5% polyacrylamide gels (41), which were fixed, treated for fluorography with Entensify (NEN, Boston, MA), dried and autoradiographed.

Cell lines

The mouse cell lines used included T lymphoma (EL4 and BW5147), macrophage (P388D1 and J774), mastocytoma (P815), B lymphocyte (M12 and A20), plasmacytoma (J558) and dendritic (D2SC1 and CB1DG) cell lines (42). Cells were cultured in RPMI 1640 medium (Gibco/BRL, Paisley, UK) containing 10% FCS (Gibco/BRL) and 5×10^{-5} M β-mercapto-ethanol.

Fluorescence in situ hybridization analysis

Fluorescence *in situ* hybridization to murine metaphase chromosomes was performed as previously described (43). The murine probe was labeled with biotinylated-11-UTP by random priming and hybridized to normal murine chromosomes. The hybridization signal was revealed by avidin-conjugated isocyanate, the chromosome counterstained with propidium iodide and R-bands prepared.

Sequence analysis

Programs such as gapped BLAST were used to search for similarities in the database banks (28). Guidelines for the use of HCA have been published previously (30,44,45). Briefly, protein sequences are shown on a duplicated α-helical net with amino acid numbers indicated above. The contours of the hydrophobic residues are automatically drawn. The α-helical net provides the best correspondence between the positions of hydrophobic clusters and regular secondary structures (46). Four symbols are used in the plots, indicating the specific structural behavior of the amino acids they represent: a star for proline, a diamond for glycine, a square for threonine and a dotted square for serine. The statistical significance of alignments is assessed by calculating *Z* scores. The *Z* score is the difference between the alignment score under consideration and the mean score of a distribution computed for the alignment of sequence 1 versus a large number (10,000) of randomly shuffled versions of sequence 2 (30,47). These differences are expressed relative to the SD of the random distribution, with values >3.0 considered to represent authentic relationships for small domains.

Detection of murine BCMA mRNA by RT-PCR

Total murine tissue RNA was purchased from Ambion. Poly(A)$^+$ RNAs from tissues and cultured cells were prepared using Quick Prep micro mRNA purification kit (Pharmacia Biotech) and then converted into single-strand cDNA using the First-Strand cDNA synthesis kit (Pharmacia Biotech). Aliquots (10 ml) of each single-strand cDNA were then submitted to PCR with primer pair no. 4/MC5′: 5′-CCCGAGGCCCTGAAGGAC-3′ and no. 2/M3′: 5′-CTAACGA-CATGTAAAACACCAG-3′, located in the second and third exons, or with the primer pair: no. 1-2: 5′-GGCGCAA-CAGTGTTTCCACA-3′ and no. 3-2: 5′-CTCGGTGTCG-GCCTTGTCCA-3′, located in the first and third exons of BCMA cDNA. The MACTIN5: 5′-GTGGCTCCATCTTGGCCTC-3′ and MACTIN3: 5′-GAAGCACTTGCGGTGCAC-3′ primer pair was used as internal control amplifying a 101 bp fragment of mouse actin mRNA. Then 5 µl of each PCR product was analyzed by agarose gel electrophoresis.

Identification of intron–exon boundaries

The junctions between exons and introns and the size of the introns of the murine BCMA gene were determined by restriction analysis of the bacterial artificial chromosome (BAC) no. 11215 clone followed by PCR amplification and nucleotide sequencing. Additional Southern genomic blots were prepared to confirm the size of restriction fragments. The primer pairs used contained sequences from opposite strands of two putative neighboring exons of the cDNA. PCR conditions included 250 µM dNTP, 150 pmol of each primer and 2.5 U of Taq DNA polymerase (Perkin-Elmer, Norwalk, CT). The PCR amplification was performed in a Perkin-Elmer 2400 thermocycler. Sequences were obtained using specific primers, and were compared with the murine BCMA cDNA to identify the coding sequences and the intron–exon boundaries.

Results

BCMA sequences

Southern blots of genomic DNA from several species were hybridized with the human BCMA cDNA probe (Fig. 1). They showed, in all mammalian samples tested, DNA sequences homologous to the human BCMA gene. The human BCMA probe detected the predicted 3 kb human fragment (lane 1), a monkey 3.1 kb (lane 2), a rat 4.1 kb (lane 3), a mouse 4.9 kb (lane 4), a dog 6 kb (lane 5), a rabbit 7 kb (lane 7), a yeast 2.6 kb (lane 9), and two distinct cow 5.8 and 1.4 kb (lane 6) fragments. The faint lower band (1.4 kb) detected in the cow DNA lane and the single band detected in the yeast DNA lane were due to non-specific hybridization. There were no signals in the non-mammalian lanes (lane 8 for chicken), and the lanes corresponding to ostrich, quail, trout, tadpole and *Drosophila* DNA (data not shown). We therefore conclude that there are genomic sequences homologous to the human BCMA gene in several mammalian species, including the mouse.

Cloning of a mouse BCMA cDNA

The murine sequences homologous to human BCMA sequences were isolated by constructing a mouse spleen

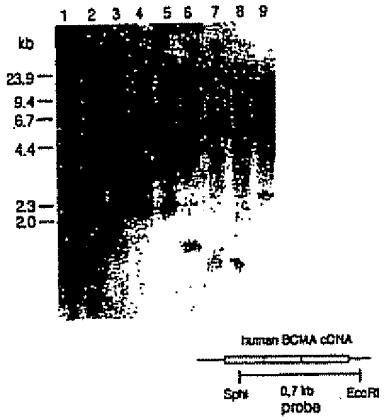

Fig. 1. Evidence for sequences homologous to human BCMA. Genomic DNA (10 μg) from human (lane 1), rhesus monkey (lane 2), rat (lane 3), mouse (lane 4), dog (lane 5), cow (lane 6), rabbit (lane 7), chicken (lane 8) and yeast (lane 9) were digested with *Eco*RI, electrophoresed on agarose gel and blotted onto a nylon membrane. The filter was hybridized with a human BCMA probe, washed under low stringency conditions and autoradiographed. The mol. wt markers are shown on the left of the blot (λ *Hin*dIII). A diagram of the human BCMA probe used is shown at the bottom of the figure.

cDNA library in the λZAPII vector and screened with the human BCMA cDNA probe under low stringency washing conditions. One 0.8 kb mouse cDNA clone was isolated and sequenced. Anchor RT-PCR experiments were used to clone and sequence the 5′ and 3′ ends of BCMA cDNA and provide its complete sequence, including the poly(A)$^+$ tail. This provided an additional 41 nucleotides on the 5′ end, and two nucleotides and the poly(A)$^+$ tail to the 3′ end of the mouse BCMA cDNA sequence.

The complete nucleotide sequence of the mouse BCMA cDNA, aligned to that of human BCMA cDNA, is shown in Fig. 2. The murine BCMA cDNA sequence has an open reading frame (ORF) of 555 nucleotides, a 144 nucleotide 5′-untranslated region and a 47 nucleotide 3′-untranslated region. It has 69.3% nucleotide identity with the whole sequence of human BCMA cDNA. The nucleotide identity between these two cDNAs is higher (73.7%) when only the coding regions are compared. The 3′-untranslated region contains no conventional polyadenylation signal. The 555 nucleotide ORF starts at position 145 with a perfect Kozak consensus sequence for the initiation codon (48) and encodes a 185 amino acid protein with a TAA stop codon at position 700. Although the murine BCMA gene, like human one, lacks an N-terminal signal peptide sequence, the presence of an upstream stop codon indicates that the complete ORF is contained within this clone. The deduced murine BCMA protein has a calculated mol. wt of 20.4 kDa and an estimated isoelectric point of 5.41. Figure 3 shows the alignment of the deduced mouse and human BCMA proteins. They are 62% identical, corresponding to a 73% similarity with only 4% gaps. The amino acid sequence of mouse BCMAp has a single hydrophobic region (residues 50–72) that has features of a membrane-spanning segment. The relative positions of the various potential post-translation modification sites in mouse and human proteins showed that only four sites were common to the mouse and human BCMA proteins: two protein kinase C (PKC) phosphorylation motifs (positions 44 and 156 on the murine sequence) and two casein kinase II (CKII) phosphorylation sites (positions 121 and 126 for the mouse protein).

The mouse BCMA cDNA insert was subcloned in a pGEM-4 vector, transcribed and translated *in vitro* with [^{35}S]methionine, and the translation product analyzed by SDS–PAGE (Fig. 4). The translation product migrated as two bands with apparent mol. wt of 20 and 26 kDa. The lower fainter 20 kDa band was probably due to an internal AUG codon. The apparent mol. wt (26 kDa) was higher than that predicted from the amino acid sequence (20.4 kDa).

Genomic organization of the murine BCMA gene

The entire mouse BCMA genomic sequences were cloned using the murine BCMA cDNA probe to screen a BAC bank (Genome Systems, St Louis, MI) and the no. 11215 BAC clone was isolated. We established a partial restriction map of the region containing the mouse BCMA gene and verified the already obtained cDNA sequence by establishing the entire coding sequence and determined the exon/intron boundaries (Fig. 5A and B). The 9.5 kb mouse BCMA gene has three exons, separated by two introns of 1 kb (intron 1) and 4.5 kb (intron 2); it is flanked by GT donor and AG acceptor consensus splicing sites (49). The human BCMA gene is similarly organized into three exons, but is only ~3 kb long, with introns of 0.75 kb (intron 1) and 1.2 kb (intron 2) (24). No canonical AATAAA polyadenylation signal was found upstream of the polyadenylation site of mouse BCMA mRNA, or downstream from this site, as the sequencing of the 3′ untranscribed region of BCMA gene showed (Fig. 5B).

Human and murine BCMA proteins and the TNFR

The FASTA and BLAST programs revealed no significant similarity for murine BCMA protein, except for the human one. HCA, a sensitive method of sequence analysis, which combines sequence comparison with secondary structure analysis, was used. This method detects sequence/structure relationships for low levels of sequence identity (below the so-called 'twilight zone'), when current one-dimension methods are not able to distinguish structural signal from background noise (30). The HCA representation of the murine BCMAp sequence is shown in Fig. 6(A). The approximate position of the transmembrane domain is readily apparent within a hydrophobic cluster of 23 amino acid residues. The N-terminal 40 amino acid domain of BCMAp contains six cysteine residues, like the 'cysteine motif' found in TNFR, in the insulin receptor and in epidermal growth factor receptor (50). The identity and similarity *Z* scores for mouse BCMAp and mouse serine protease PC6B (51), were 7.1 and 7.3 (40% sequence identity on 30 amino acids). The human BCMAp and mouse PC6B *Z* scores were 7.3 and 7.4 (38% sequence identity on 31 amino acids). These values are well above the threshold of 3, and indicate a genuine link between the BCMAp N-terminal sequences and TNFR cysteine motifs; values of 3–6 are frequently observed for divergent but related

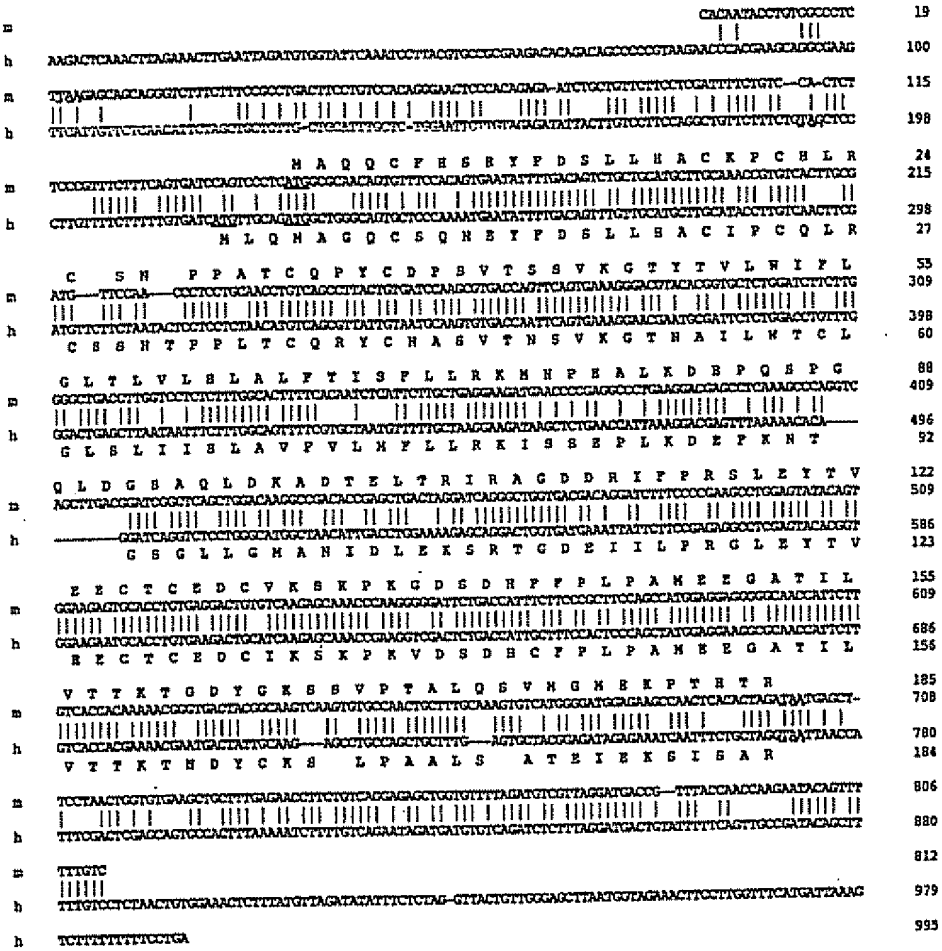

Fig. 2. Alignment of mouse and human BCMA cDNAs. Potential translation initiation ATGs are underlined with continuous lines, while codon stops are underlined with dashed lines. The deduced amino acid sequences for mouse (m) and human (h) proteins are shown in the single letter code. GenBank Accession number for mouse WNA: AF061505.

domains. Similar results were obtained for the *Drosophila melanogaster* furin-like protease (52) (31% sequence identity on 41 amino acids) and the TACI protein (53) (31% sequence identity on 36 amino acids) which belong to the TNFR superfamily. BCMAp possesses most of the other features of TNFR including the conserved aromatic residue four to six residues C-terminal from the first cysteine (4,54). BCMAp has a tyrosine at five and a phenylalanine at six residues from the first cysteine.

Expression of murine BCMA mRNA

Total RNA (10 µg) from tissues or cells was checked for BCMA murine mRNA by the RNase protection method (data not shown). Murine BCMA mRNA was found only in the spleen, bone marrow, heart and lungs (faint). Only the murine plasmacytoma cell line J558 actively expressed the BCMA gene.

BCMA mRNA was also detected by RT-PCR using primer pairs from the second and third exons of BCMA gene (Fig. 7). BCMA mRNA was also detected as a single band of 404 bp in kidneys and thymus but not in the liver, brain, testis, ovaries and embryonic tissues. BCMA mRNA was detected in murine J558 plasmacytoma, EL4 and BW5147 T cell lymphoma, A20 B cell lymphoma, and CB1D6 and D2SC1 dendritic cell lines. We cannot evidence the presence of murine BCMA mRNA in P815 mastocytoma, J774 macrophage and M12 B cell lymphoma cell lines.

When we used, as amplification primers, a primer pair located in the first and the third exon of BCMA gene, we evidenced the presence of two amplified bands, one of

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H   MLQMAGQCSQNEYFDSLLHACIPCQLRCSSNTPPLTCQRYCNASVTNSVK   50
      I II   IIIIIIIIII II IIII   II III II  III III
M   ---MAQQCFHSEYFDSLLHACKPCHLRCSN--PPATCQPYCDPSVTSSVK   45

H   GTNAILMTCLGLSLIISLAVFVLMFLLRKISSEPLKDEFKN----TGSGL   96
      TT  TT TTT I  III I   IIIII   I IIII        II
M   GTYTVLMIFLGLTLVLSLALFTISFLLRKMKPEALKDEPQSPGQLDGSAQ   95

H   LGMAWIDLEKSNTGDEIILPRGLEYTVEECTCEDCIKSKPKVDSDHCFPL   146
      I  I   I  I II  I II IIIIIIIIIIII IIIII IIII III
M   LDKADTELTRERAGDDRIFPRSLEYTVEECTCEDCVKSKPKGDSDNFFPL   145

H   PAMEEGATILVTTKTNDYCKS-LPAAL-SATEIEKSISAR             184
      IIIIIIIIIIIIIII II II  I II I   II   I
M   PAMEEGATILVTTKTGDYGKSSVPTALQSVMGHEKPTHTR             185
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Fig. 3. Alignment of the predicted mouse BCMA protein sequence with that of human BCMAp. The potential transmembrane segment is underlined. The conserved six-cysteine residues of the N-terminal are shown by an asterisk, while the three conserved cysteine residues of the C-terminal are denoted by a large dot.

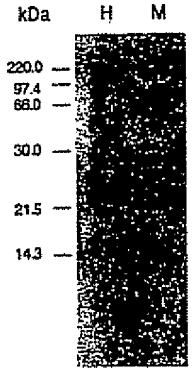

Fig. 4. *In vitro* transcription and translation of human and mouse BCMA proteins. pGEM4BCMA human (H) and pGEM4BCMA mouse (M) vectors (1 μg) were transcribed and translated *in vitro* according to the instructions of the manufacturer. The translation products were heat denatured and analyzed by SDS–PAGE on a 15% gel.

304 bp corresponding to the normal BCMA mRNA sequence and a second one of 145 bp. We have cloned these two bands and established their nucleotide sequence. The short amplified sequence corresponds to an alternate splicing, which deletes the entire second exon encoding the transmembrane region of BCMAp.

Chromosomal localization of mouse BCMAp

The chromosomal localization of the BCMA gene was determined by fluorescence *in situ* hybridization analysis using a mouse BCMA cDNA biotinylated probe, as previously described (55). Under the conditions used, mouse BCMA gene was assigned to the long arm of chromosome 16, region 16B3 (Fig. 8). This region corresponds to the counterpart of human 16p13 region where the human BCMA gene is localized (56).

Discussion

We have previously cloned and characterized a novel human gene, BCMA, which is preferentially expressed in B lymphocytes. At that time the absence of significant similarity of this gene with known sequences as well as the absence of known motifs did not help us to postulate a valuable hypothesis about the functional role of this gene. We have decided to clone the mouse homolog of the human BCMA gene in order to define the conserved regions and if possible identify any significant functional motif. In this paper we report the cloning of the murine BCMA cDNA, the isolation and characterization of the genomic BCMA fragment, the establishment of its chromosomal localization, and the study of its tissue and cell line expression. Furthermore, the alignment of the two human and murine sequences completed by HCA allowed us to define BCMA as a member of the nerve growth factor receptor (NGFR)/TNFR superfamily.

Sequences homologous to the human BCMA gene were found in all mammalian species tested (rhesus monkey, rat, mouse, dog, cow and rabbit) but not in chicken, yeast or in genomic DNA from ostrich, quail, trout, tadpole and *Drosophila*. Murine BCMA gene is 9.5 kb long and lies on murine chromosome band 16B3, the syntenic region of human 16p13 chromosome band (56). The murine BCMA gene is organized like the human one with three exons, but the second murine intron (4.5 kb) is longer than the human intron 2 (1.2 kb). The murine BCMA cDNA encodes a 185 amino acid protein (184 amino acids for the human) containing a potential transmembrane region as does the human protein (23,25). The human cDNA contains two putative transcription start codons surrounded by a Kozak consensus site but only the second is present in the murine sequence, suggesting that this second ATG codon is effectively used by the transcription machinery. Human BCMAp becomes inserted into canine microsomes as a type I integral membrane protein *in vitro*. The murine BCMAp is probably also a type I transmembrane protein. The very asymmetric distribution of charged residues in murine BCMAp (27.5% of residues of the hydrophilic C-terminus are charged, and only 12% in the hydrophilic N-terminus) suggests that the C-terminus is oriented towards the cytoplasm (57,58). Human BCMAp has several potential post-translation modification sites, but only four sites are common to human and murine proteins: one PKC phosphorylation site in the N-terminal part of the protein, and one PKC and two CKII phosphorylation sites in the cytoplasmic region. This suggests that the three cytoplasmic conserved modification sites may be used *in vivo*. The potential *N*-glycosylation site in the human sequence at position 42 is not present in mouse BCMAp, in agreement with previous results showing that human BCMAp is not glycosylated *in vivo* (25).

The human and murine BCMAp sequences have no immediate similarities with any published motif or protein. However, a cluster of six cysteines is very well conserved in their N-terminal region. These six cysteine residues can form the motif found in the extracellular domain of TNFR proteins (4). TNFR are generally type I transmembrane proteins that have two to six of these perfect or imperfect cysteine-rich domains in their N-terminal region. Human BCMAp is also a type I transmembrane protein as is, probably, the murine protein.

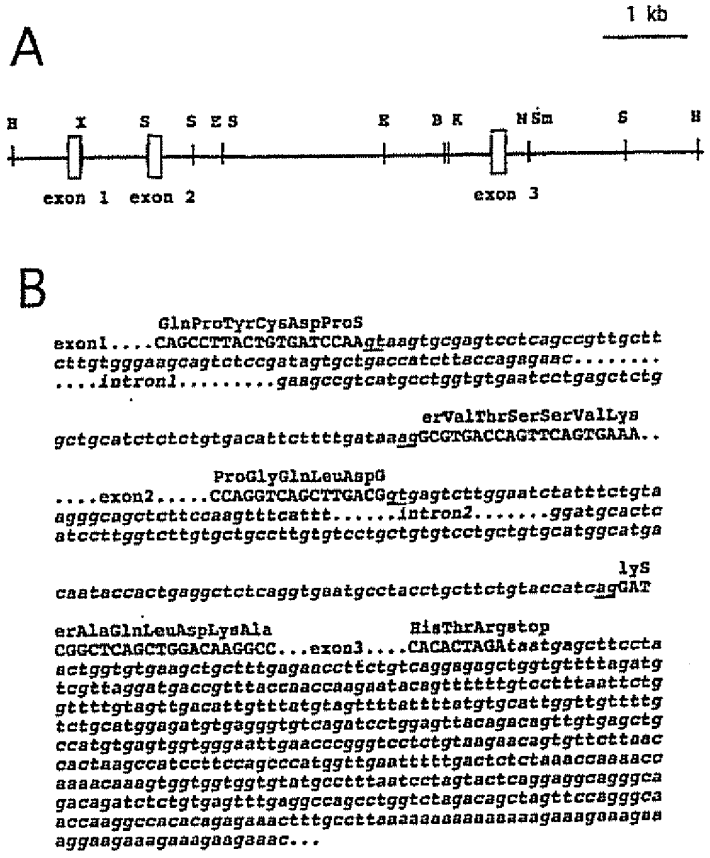

Fig. 5. Restriction map of murine BCMA gene. The partial restriction map of the murine BCMA gene is shown in (A). Exons are represented as open boxes. Restriction enzymes: *Hin*dIII, H; *Bam*HI, B; *Sac*I, S; *Nco*I, N; *Sma*I, Sm; *Kpn*I, K; *Eco*RI, E; *Xho*I, X. The exon borders' nucleotide sequences are shown in (B). The donor and acceptor splicing sites AG and GT are underlined, while the deduced amino acid sequence is shown in the three letter code.

BCMAp is the second TNFR to be reported (the other is cfECP1, a fungal TNFR) that contains a short N-terminal domain with a single cysteine motif. All TNFR proteins also contain an aromatic amino acid residue four to six residues after the first cysteine. Both human and murine BCMAp have a tyrosine five residues and a phenylalanine six residues after the first cysteine. We have therefore identified a new TNFR with a single cysteine motif.

We have also identified the reason for which BCMAp is not recognized as having a TNFR cysteine motif. The TNFR motif of the human and mouse BCMAp does not exactly match the PROSITE TNFR/NGFR family cysteine-rich region signature, {PDOC00561: C-*x*(4,6)-{FYH}-*x*(5,10)-C-*x*(0,2)-C-*x*(2,3)-C-*x*(7,11)-C-*x*(4,6)-{DNEQSKP}-*x*(2)-C}. This is too strict for the sequence separating the two last cysteines (loop 3: underlined). BCMAp differs from the classical TNFR domains, as described by the PROSITE signature, in the C-terminal part of the motif. BCMAp may have a shorter loop 3 with a disulfide bond pattern similar to that of classical TNFR motifs or a second sub-module that is different from the B2 one, perhaps similar to the C2 one of the fourth cysteine motif of TNFR in which the two last cysteines are also separated by three residues (6). This is also true for the TACI protein.

The cytoplasmic tails of TNFR are generally dissimilar, indicating the great variety of signals transmitted. The BCMAp C-terminal region is unlike that of any other TNFR. It does not contain a death domain motif, which leads to apoptosis upon ligand binding (9,10). Neither the murine nor the human BCMA protein contains either of the two known motifs that recruit the cytoplasmic proteins TRAF1, 2 or 3 (59). However, the remarkable conservation of the membrane proximal region in the mouse and human BCMAp (93% identity at the amino acid level) indicates that this internal part could play a role in signal transduction through the binding of specific effector proteins.

Human BCMA mRNA is expressed preferentially in

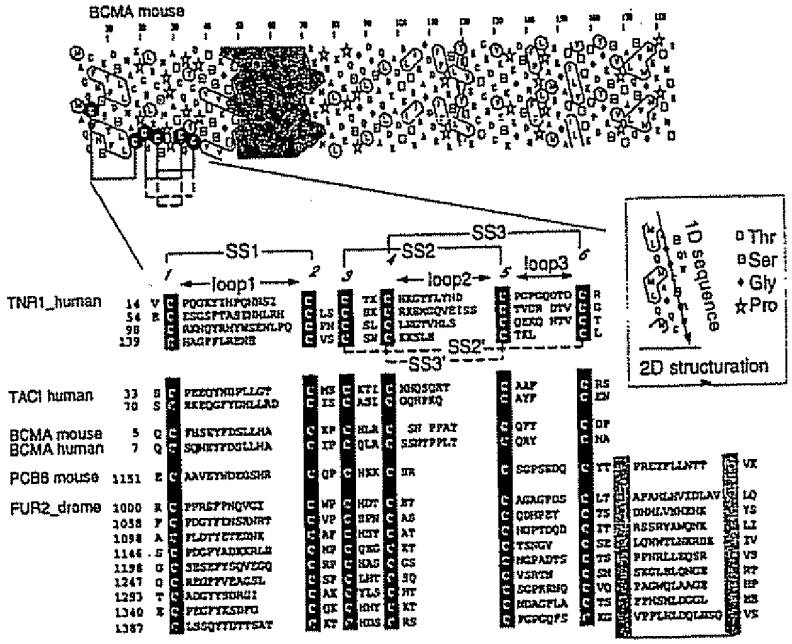

Fig. 6. (A) HCA representation of the mouse BCMAp sequence. The protein sequence is shown on a duplicated α-helical net with amino acid numbers indicated above it. The symbols used in the plot and the manner in which the one-dimensional sequence and the two-dimensional structures are presented are indicated in the insert. The transmembrane region is shaded grey. The N-terminal region contains six cysteine residues (white letters) that are conserved in the human BCMAp sequence and which define a characteristic TNFR cysteine motif. The potential disulfide connections are shown as deduced from the three-dimensional structure of the first three domains of TNFRI (solid line) or from the three-dimensional structure of the fourth TNFRI domain (dashed line). (B) Alignment of the single cysteine motif of mouse and human BCMAp with the cysteine motifs of TNFRI (SW: TNR1_human), *Drosophila melanogaster* furin-like protease (Sw: FUR2_drome), one of the cysteine motifs of the mouse PC8B serine protease (Pir: S34583) and the two motifs of the human TACI (GenBank: AF023614).

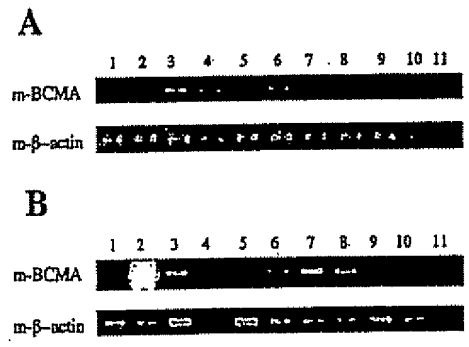

Fig. 7. Detection of mouse BCMA mRNA in tissues and cultured cell lines. Poly(A)$^+$ mRNA (1 μg) was reverse transcribed and subsequently amplified by PCR using a primer pair located in the second and third exons of mouse BCMA mRNA. (A) Mouse tissues, liver (1), brain (2), thymus (3), heart (4), lung (5), spleen (6), testis (7), ovary (8), kidney (9), embryo (10) and none (11). (B) Cultured mouse NIH3T3 (1), J558 (2), A20 (3), M12 (4), EL4 (5), BW5147 (6), CB1DG (7), D2SC1 (8), J774 (9), P815 (10) and none (11) cell lines. The control mouse β-actin mRNA is also shown.

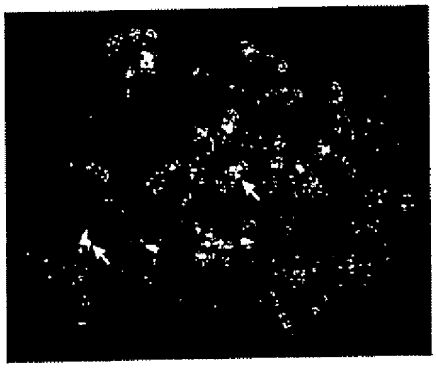

Fig. 8. Chromosomal location of the murine BCMA gene. Fluorescent *in situ* hybridization to murine R-banded metaphase chromosomes. Two signals are visible on each chromatid chromosomes 16 (band 16B3).

lymphoid organs and B lymphocytes (24,25). We found, by using RNase protection, that the murine BCMA gene is faintly expressed in lymphoid organs and not expressed in

lymphocyte cell lines, except the plasmacytoma J558 cell line, where it is very active. RT-PCR detected murine BCMA gene expression that is similar to that of the human and it seems to be specific to secondary lymphoid organs. Low expression of BCMA gene activity was found in several lymphocyte cell lines. There is also at least one variant species of BCMA mRNA, expressed in mouse spleen, due to alternative splicing of the second exon. Alternative splicing products are common in TNFR.

The TNFR can mediate, upon ligand binding, the action of protein factors leading to cell death or cell proliferation and differentiation. BCMAp is mainly expressed in all tissues in which lymphoid cells are enriched. This expression pattern suggests that BCMAp plays a role in the development and regulation of the immune system. Identification of its cognate ligand will greatly improve our understanding of the physiological role of BCMAp.

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Abbreviations

BAC	bacterial artificial chromosome
BCMA	B cell maturation
BCMAp	B cell maturation protein
CKII	casein kinase II
HCA	hydrophobic cluster analysis
NGFR	nerve growth factor receptor
ORF	open reading frame
PKC	protein kinase C
TNF	tumor necrosis factor
TNFR	tumor necrosis factor receptor
TRAF	TNFR-associated factor

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